WS15.1 Volume increase in alveolar type II cells following fusion dependent activation of P2X4 receptors on lamellar bodies – linking secretion and fluid transport in the lung?

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Maintenance of a healthy lung environment relies on proper fluid transport within the lungs. While it has been thought that the alveolar epithelium may play a part in regulating fluid homeostasis, this has remained largely undetermined. The alveolar epithelium consists of the alveolar type I and II (ATII) cells. ATII cells are responsible for secretion of lung surfactant through exocytosis of lamellar bodies (LBs). Recently we have described that exocytic fusion of LBs with the plasma membrane results in a non-selective, transient, and inward-rectifying, cation current. This inward-cation current occurs across the purinergic receptor, P2X4, which is located on the membranes of fused LBs (PNAS 2011, 108(35):14503–8). It is possible that directed ion transport across the ATII cell results in either fluid secretion or absorption into or from the pulmonary hypophase. This leads to the possibility that fluid transport across the alveolar epithelium is modulated by this fusion-activated cation influx following LB exocytosis. Atomic force microscopy (AFM) and fluorescence experiments confirmed that exocytosis of LBs following ATP stimulation results in an instant increase in ATII cell volume, and is then regulated within minutes. In ATII cells with LBS fusing with the plasma membrane following ATP stimulation, there was a 30% increase in cell height following fusion. This effect was not seen however in ATII cells without fusion of the LBs with the plasma membrane. These data indicate a coupling of surfactant secretion (via LB fusion) and the regulation of fluid transport in the lung.

WS15.2 Airway surface liquid layer height in cystic fibrosis bronchial epithelial cells is increased by lipoxin A_4 via an apical ATP release activating a P2Y receptor pathway

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Cystic fibrosis (CF) is caused by a mutation of the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) gene. One of the resulting abnormalities is dehydration of the Airway Surface Liquid (ASL) by a dysfunctional ion transport, leading to impaired mucocillary clearance, lung infection and inflammation. The eicosanoid LXA_4 is a signal of the resolution of inflammation, and is decreased in the lungs of patients with CF (Karp et al, 2004). The decrease in LXA_4 could participate to chronic airway inflammation in CF. Using live cell imaging, we investigated the role of LXA_4 on ASL height in Human Bronchial Epithelial Cells (HBE) and Human Cystic Fibrosis Bronchial Epithelial (HCFBE) cells cultured in air/liquid interface. LXA_4 and its stable analogue TA39 increase ASL height in a dose and time dependent manner in both the HBE and HCFBE cells. LXA_4 causes apical ATP release from HBE and HCFBE cells which was inhibited by a Panx1 inhibitor, carbenoxolone. Inhibition of the P2Y11 receptors with reactive blue 2 and NF340, prevents the ASL height increase induced by LXA_4. The ASL height increase and ATP release induced by LXA_4 were both blocked by the FPR2 receptor inhibitor, Boc-2. Our results indicate a novel role for LXA_4 in restoring ASL height in CF airways via a mechanism involving the FPR2 receptor, apical ATP release and P2Y11 receptor activation. This could lead to a new therapeutic route for CF patients.

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WS15.3 Channel activating proteases (CAPS): identification of novel targets for correcting sodium channel dysfunction in cystic fibrosis

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Proteolytic cleavage of the endogenous α and γ subunits of ENaC [by channel activating proteases (CAPS)], is a key regulatory mechanism for ENaC channel activity. Excessive ENaC activity is a feature of CF which contributes to the depletion of the ASL (airway surface liquid) with resultant impairment of mucociliary function. Growing evidence suggests protease/protease inhibitor imbalance may underlie increased Na^+ absorption in CF airway epithelia therefore inhibition of the CAPS-ENaC signalling axis represents a potential therapeutic target to restore mucociliary function.

Our group has developed a panel of active-site directed affinity probes which target and inhibit trypsin-like proteases (potential CAPS) including the broad-spectrum inhibitor QUB-TL1. QUB-TLI selectively inhibits recombinant trypsin-like proteases (trypsin, prostasin, furin) without affecting non-trypsin like proteases (neutrophil elastase); and furthermore inhibits trypsin-like protease activity in cell-conditioned media (CCM) retained from epithelial cell cultures. By targeting the biotin reporter group on QUB-TL1 with anti-streptavidin-HRP we can visualise several candidate CAPS in

1. intact non-CF and CF epithelial cells (HBE/CFBE cell lines and primary nasal epithelial cells); and
2. CCM from these epithelial cell cultures.

Conclusion: Our broad-spectrum affinity based probe detects several trypsin-like species in non-CF and CF epithelial cell preparations. This paves the way for further characterisation of these proteases and an assessment of their effect on ENaC function.

WS15.4 Study of the chloride channel ANO1 in cystic fibrosis context

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Defective CFTR function in the airway epithelium is responsible for CF patient lung disease. CFTR represents the most important pathway for chloride (Cl^-) secretion in human bronchial epithelial cells. Calcium activated Cl^- channels (CaCC) are also an important pathway of Cl^- secretion. In 2008, three teams identified ANO1 (Anoctamine 1) as a CaCC candidate. The main aim of this study is to characterize ANO1 protein in CF vs non CF context. This aim is divided into three distinct parts that are intended to:

- Compare ANO1 expression, localization and activity in CF vs non CF context
- Understand mechanisms regulating ANO1 activity
- Determine whether there are differences between CF and non CF alternative splicing

Our results show that ANO1 expression and activity are significantly decreased in CF models (cell lines, mice and lung explants). ANO1 is expressed at plasma membrane in CF and non CF cells. Preliminary results shows that ANO1 activity is reduced in presence of Mek1,2 inhibitors. Alternative splicing study shows that expression of some exons seems to be different between CF and non CF models.

We conclude that decreased ANO1 activity in CF cells could be explained by decreased ANO1 mRNA and protein expression and may contribute to the worsening of ionic imbalance. Moreover, ANO1 seems to be regulated by Erk1,2 pathways although these results should be confirmed. First results obtained on alternative splicing seem to show that CF or non CF context induces different alternative splicing that could affect ANO1 function. All of these results lead us to think that this Cl^- channel could be a potential pharmacological target for the treatment of CF patients.